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In vitro Study of Transvascular Transport of Nanostructures using a 3D Printed Microfluidic Platform

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Introduction

In recent years, gold (Au) nanoparticles have attracted growing interest as nanoscale diagnostic and therapeutic agents owing to their unique set of physical, chemical, electrical and optical properties [1]. Compared with conventional nanoscale agents, Au nanoparticles have well defined and dense surface structures, which allows higher load of drugs, surface agents, ligands, etc [1]. Using various Au nanostructures as site-specific cancer drug delivery has been demonstrated to increase the drug concentration in the tumor while minimizing systemic toxicity because Au nanoparticles preferably accumulate at the tumor sites after intravenous injection. As tumors have dense and leaky vasculature, the circulating nanoparticles preferentially extravasate through the larger pores in the capillary wall into the tumor tissue [2]. After extravasation, the particles are less likely to be recirculated due to the lack of a lymph system in the tumor and low diffusivity of the nanoparticles. The current challenge facing the site-specific drug delivery is the low targeting efficacy: only a fraction of the injected nanoparticles finally reaches the targeted site. The transvascular transport of the nanoparticles in tumors presents a major barrier for nanoparticle delivery to the target site. The permeability of the targeted tumor's vasculature, the tumor interstitial pressure, and nanostructure parameters, such as size, shape, surface properties, and concentration within the blood stream, are important factors that dictate the payload of nanoparticles delivered to the targeted site. It is of critical importance to assess the transvascular transport behavior of the nanoparticles in the development of nanoscale agents with novel composition, structure and surface properties.

Objective

The objective of this study is to investigate the transvascular transport behavior of in house developed Au spherical nanoparticles and nanorods using a simplified microfluidic platform. The in vitro model is used to study the effect of the shape of the nanoparticles and interstitial pressures on the number of nanoparticles penetrating micronized pores.

Material and Method

Design of the device: As illustrated in Fig. 1 (a), the designed microfluidic device consists of a microchannel embedded in the top PDMS layer and a chamber situated in the bottom PDMS layer. A piece of polycarbonate porous membrane with a pore size of 1 micron is embedded between the top and bottom layers to mimic the capillary wall. The microchannel is15 mm in length, 0.4mm in width, and 0.12mm in depth, while the chamber in the bottom is 2.3mm long, 6 mm wide, and 0.12mm in depth. The liquid pressure in the channel and the chamber are controlled separately by the elevations of the fluid reservoirs connecting with the channel/chamber. The pressure difference between the channel and the chamber results in a flux of the nanoparticle-loaded liquid crossing the membrane. Some nanoparticles may also diffuse across the membrane due to the concentration difference between the channel and the chamber. The microchannel and chamber are made using soft lithography method. The mold with negative design of the microfluidics as shown in Fig. 1 (b) is created using the commercial software, SOLIDWORKS.

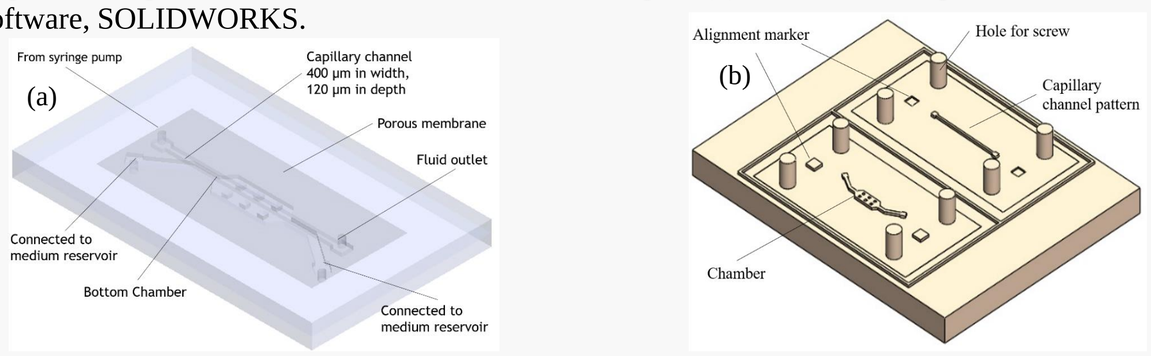

Figure 1. Illustration of the microfluidics design and CAD model of the mold of the microfluidics device with negative design pattern. Fig. 1(a) Microfluidics design. Fig. 1(b) CAD model of the mold

***Fabrication of the mold*:** The molds for the microchannel and microchamber are printed using an SLA 3D printer (Formlabs Form2, Somerville, MA). To ensure a smooth surface, a highest resolution of 40 microns was used. The mold wall thickness is 5 mm to prevent warpage during PDMS curing process.

Casting and curing of the PDMS platform: 36 grams of PDMS base was homogenously mixed with 3.6 grams of the curing agent at a weight ratio of 10:1 (SYLGARD 184 SILICONE ELASTOMER, Ellsworth Adhesives, WI). Then, 39.6 grams (40 ml) of the mixture was poured onto the molds fixed in a 100 mm dia. petri dish. After degassing in a vacuum chamber (RS – 1, Best Value Vacs, Illinois) for an hour, the petri dish was placed on a piece of fire brick in an oven (10GC, Quincy Lab, Inc., Illinois) preheated to a setup temperature of 65℃. After curing in the oven for an hour, the PDMS part was peeled. Inlet and outlet port of PDMS were punched using the biopsy punch (Electron Microscopy Sciences, Hatfield, PA).

***Assembly of the PDMS layers with porous* membrane:** The two-layer microfluidic device is assembled by bonding the PDMS layers with a piece of polycarbonate membrane sandwiched in between [3]. The porous membrane having a pore size of 1 micron a (Sterlitech Inc Kent, WA) was first activated in the oxygen plasma (100W, 500m torr) for 1 minute. Then it was immersed into the 2% volume of water diluted (3-Aminopropyl) triethoxysilane, APTES solution(Sigma-Aldrich, St Louis, MO) at 80℃ for 20 minutes. The top and bottom PDMS layers were also activated in the oxygen plasma (100W, 500m torr) for 20 seconds. The assembled microfluidic device was further heated at 60℃ for 24 hours to ensure a strong bonding. After the device was assembled, the bottom chamber was connected to the deionized water reservoir maintained with constant water height as shown in Figure 2a. The inlet of the microchannel was connected to a syringe pump that allows controllable injection rate, while the pressure at the outlet of the microchannel is controlled by adjusting the elevation of the discharging tube. After flushing the microchannel, the microchamber, and the tubing with the deionized water, the platform was filled with water to ensure there were no leakage and air bubbles in the system. 1mL of nanoparticles/nanorods was then injected into the microchannel at a flowrate of 1μL/min. Injection lasted for 16 hours. We tested Au Fluorescein isothiocyanate, FITC spherical nanoparticles (GNP) with a diameter of 17 nm at a concentration of 5.67×10^{-10} M and an intensity of 1.17×10^{6}, and Au nanorod FITC (GNR) with a diameter of 15 nm and a length of 48 nm at a concentration 8.50×10^{-10} M and a intensity of 8.46×10^{6}. The chemical structure of synthesized Au nanoparticles and nanorods is shown in Fig. 2(b). In this experiment, we tested injection of nanoparticles/nanorods under two pressure differences between the channel and the chamber: 20 mmHg and 5 mmHg pressure. Each test was repeated three times, and a total amount of 12 tests were conducted. After a test, the liquid from the bottom chamber was withdrawn with a syringe and the volume of the water was measured. The total volume of the liquid in the bottom chamber and the tubing was measured to be 200 μL by using micropipette (Fisher brand, Huston, TX).

(a)

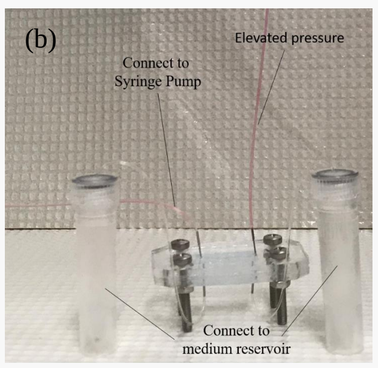

Figure 2. The chemical structure of GNP and GNR, and the experimental set up. Fig. 2(a) Chemical structure of GNP and GNR. Fig. 2(b) experimental set up

Quantification of the nanoparticles/ nanorods concentration: The absorbance of the liquid containing nanoparticles/nanorods taken from the bottom chamber was inspected using ultraviolet–visible spectroscopy (SE3300 UV-VIS Spectrophotometer, Azzota Corporation, Claymont, DE) and the fluoresce intensity of the same mixture was inspected using Fluorometer (FluoroMax-3, Horiba Jobin Yvon, Edison, NJ). From spectrophotometer, the wavelength and Au absorbance in water data was obtained. By using Beer-Lambert's law, the concentration of Au nanoparticles in solution was calculated [4]. The concentration of GNP and GNR liquid mixture can be determined by the method described in the reference [4]. Once the concentration of is determined, the total number of GNP and GNR can be calculated.

Results and Discussions

The fraction of GNP/GNR crossing the membrane under two pressure differences is shown in Fig. 3. For spherical Au nanoparticles, the averaged fractions that permeate across the membrane under 20 and 5 mmHg are 0.40 and 0.29, respectively. The averaged fractions of Au nanorods that permeate across the membrane under the same pressure differences are 0.5 and 0.38, respectively. The results show that in addition to the pressure difference between the microchannel and the chamber, the elongated shape of the nanorods also facilitate the permeation of the nanostructures across the membrane. Under an identical pressure difference, a higher fraction of Au nanorods permeate across the membrane than spherical nanoparticle. This is consistent with previous in vivo study [6] that demonstrated a higher flux of Au nanorods extravasating the tumor vasculature than the nanoparticles. Moreover, more Au nanorods visibly deposit on the membrane after each test as shown in Fig. 4. This observation can be explained by the Liu et al.'s [7] numerical study that confirms that nanorods are prone to attachment on the vasculature wall. We consider that the high deposition rate of Au nanorods on the membrane causes particle aggregation, and in the worst case, the blockage of the pores of the membrane.

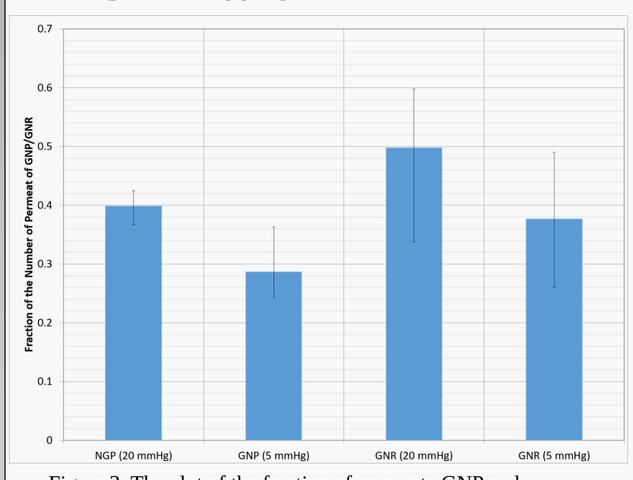

Figure 3. The plot of the fraction of permeate GNP and GNR under 5 mmHg and 20 mmHg pressure.

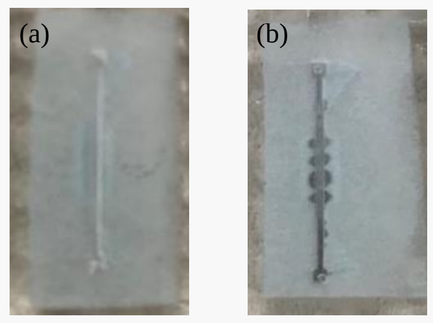

Figure 4. Picture of the membrane after the experiment under 20 mmHg. Fig. 4(a) GNP experiment. Fig. 4(b) GNR experiment.

Conclusions

Microfluidics platform simulating gold nanostructures transport in tumor vascular was fabricated using 3D printing technology. Synthesis Au nanoparticles and nanorods are used to test the fabricated device using 1μm porous membrane under 20 mmHg and 5 mmHg capillary channel pressure condition. Higher capillary channel pressure has results in higher permeate of particles and rods across membrane. Compare to spherical shape nanoparticles, nanorods structure has higher permeation. Aggregation and blockage occurred around the membrane for nanorods experiment.

Reference

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